Effect of Barley Grass Juice on Antioxidant Capacity and DNA Damage in Diabetic Rats

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Abstract

Objective: Worldwide, phytotherapy methods acquire great importance, and studies in this field are increasing their importance each day. This study, it was aimed to examine total antioxidant, oxidant status, oxidative DNA damage, glucose and hemoglobin A1c levels and the effects of barley grass juice on these parameters in rats with diabetes mellitus.

Material and Method: Four groups were formed for the study and 6 male rats weighing 250-350 g were used in each group. Control Group; physiological saline was injected via intraperitoneal., Diabetic Group; created using streptozotocin, Barley Grass Group; Barley grass juice (3 ml/rat/day) was given orally for 4 weeks, Diabetic and Barley Grass Group; was injected streptozotocin and barley grass juice was given by oral for 4 weeks.

Results: It was determined that barley grass juice decreased blood sugar, glucose, hemoglobin A1c, total oxidative status and oxidative stress index values, increased total anti-oxidative status value, and body weights in streptozotocin-induced diabetes. In addition, it was determined that the addition of barley grass juice provided a significant protective effect and improvement in these parameters.

Conclusion: Based on these findings, we can say that barley grass juice has an antidiabetic-antioxidant effect and weight gain in diabetes mellitus

Keywords: Antioxidant, Barley grass juice, Diabetes mellitus, DNA damage, Glucose, HbA1c, Oxidan

Özet

Amaç: Dünya çapında fitoterapi yöntemleri büyük önem kazanmakta ve bu alandaki çalışmalar her geçen gün önemini artırmaktadır. Bu çalışmada deneysel olarak diyabet oluşturulan ratlarda toplam antioksidan, oksidan durum, oksidatif DNA hasarı, glikoz ve hemoglobin A1c düzeyleri ve arpa çimi suyunun bu parametreler üzerine etkisinin incelenmesi amaçlandı.

Gereç ve yöntem: Çalışma için 4 grup oluşturuldu ve her grupta 250-350 gr ağırlığında 6 erkek rat kullanıldı. Kontrol grubu; serum fizyolojik periton içi yol ile enjekte edildi, Diyabet grubu; diyabet, streptozotosin kullanılarak oluşturuldu, Arpa çimi grubu; 4 hafta oral olarak arpa çimi suyu (3 ml/sıçan/gün) verildi, Diyabet+Arpa çimi grubu; streptozotosin ile diyabet oluşturuldu ve 4 hafta boyunca arpa çimi suyu oral olarak verildi.

Bulgular: Arpa çimi suyu, streptozotosin kaynaklı diyabette kan şekeri, glikoz, HemoglobinA1c, toplam oksidatif durum ve oksidatif stres gösterge değerlerini düşürdüğü, toplam antioksidan değerini ve canlı ağırlıkları arttırdığı belirlendi. Ayrıca arpa çimi suyu ilavesinin bu parametreler üzerinde belirgin koruyucu etki ve iyileşme sağladığı tespit edildi.

Sonuç: Bu bulgulardan yola çıkarak arpa çimi suyunun şeker hastalığında anti-diyabetik, anti-oksidan etki gösterdiği ve kilo kaybını önlediğini söyleyebiliriz.

Anahtar Sözcükler: Antioksidan, Arpa çimi suyu, Diyabet, DNA hasarı, Glikoz, HbA1c, Oksidan

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Introduction

Barley and extracts contain 30 rich ingredients. Six components of barley grass (flavonoids, Gamma-aminobutyric acid, potassium-calcium, superoxide dismutase, tryptophan, and vitamins) are very important. These components can fight more than 20 chronic diseases (1).

Barley grass has been reported to be a very powerful plant used in the prevention of hypertension with its high calcium, potassium, magnesium, and low sodium values. However, one of the factors affecting the formation of cancer is that the pH of the environment shifts towards acidity, and the content of barley grass is an alkaline pH, so it can be used in cancer treatment (1).

Barley grass (BG) contains a wide variety of nutrients and plant hormones, antioxidants (superoxide dismutase, catalase, vitamin E, vitamin C, and carotenoids), and up to 3000 enzymes used by the body. BG has alkaline content, so it can reduce intense acidity, which has an important effect on cancer development (2).

Vitamin E is one of the fat-soluble antioxidant vitamins. There are 4 types of vitamin E in barley, α-tocopherol, α-tocotrienol, β-tocopherol and β-tocotrienol (3). Vitamin E in barley is 0.850-3.15 mg/100 g, and its antioxidant capacity is between 57.2 and 158.1 mg/100 g (4).

The balance between antioxidant defense and free radical production in the organism is very important for health. If there are too few free radicals or oxidants, this can lead to chronic permanent damage. The oxidant or antioxidant components can be measured separately, but this process is time-consuming and costly. Thus, total oxidative status (TOS), antioxidant status (TAS) and oxidative stress index (OSI) measurements reflect this situation and they are more economical and practical (5,6).

It is known that reactive oxygen species (ROS) are generally effective in various physiopathological conditions. ROS production can cause DNA damage that leads to chronic health problems. This can be observed in diseases such as cancer, aging, and chronic inflammation (7). Recently, 8-hydroxydeoxyguanosine (8-OHdG) has been used in laboratory analysis as a parameter of oxidative DNA damage.

Diabetes Mellitus (DM), is a chronic disease, it causes disorders of carbohydrate, fat and protein metabolism. Complications that may develop with the disease are microvascular, macrovascular, and neuropathic.

The organism has an antioxidant defense system. However, due to the increase in oxidative stress in some metabolic diseases such as diabetes, an increase in free radicals also occurs. This may cause loss of membrane integrity and genetic mutations; these effects of oxidants can be overcome by the application of antioxidants (8).

Streptozotocin (STZ) (9,10) and alloxan (11,12) are used in in vivo studies to induce DM in experimental animals. STZ is preferred in vivo studies because it causes beta cell cytotoxicity (13).

In recent years, although studies are examining the effect of BG on nephrotoxicity (14), wheatgrass on renal failure (15) and diabetes (10); the antioxidant effect of borax (16), boric acid (17), resveratrol (18,19) there is no study about the effect of barley grass on TOS, TAS, and DNA damage glucose and hemoglobin A1c (HbA1c) in diabetic rats. Therefore, we investigated the effects of barley grass on these parameters.

Material and Method

Chemicals: physiological water with saline (% 0,09) was provided from Polifarma (Istanbul, Türkiye). STZ was obtained from Sigma-Aldrich Chemical Company. Anesthetic agents were purchased from Pfizer (Ketalar), and Bayer (Rompun). Barley grass was obtained from barley seed at 7 days under suitable conditions in a hasılmatik machine. The barley grass was crushed in the juice machine and the juice was obtained. Barley grass juice was given to rats, fresh.

Animals: In this study, a total of 24 Wistar albino male rats were used and their weights were between 250 and 350 g. Animals were obtained from Yuzuncu Yil University Experimental Animal Center. The animals were kept in an animal care center with climate control, where they were kept in rooms with 12 hours of light/dark, a temperature of 24± °C, and a relative humidity of 45±5 % and in non-condensed plastic cages. To ensure the animals' adaptation standard rat chow and water were given ad libitum 7 days before and throughout the study. Ethical approval for this study was approved by the local ethics committee of Van Yuzuncu Yil University in Turkey (2015/14). In addition, research and publication ethics were complied.

Experimental protocols: The experimental groups were formed as follows.

The control (C) group; was injected with isotonic saline intraperitoneal (i.p.).

Barley Grass (BG) group; was given BG juice (3 ml/rat/day) orally for 4 weeks.

Diabetes Mellitus (DM) group; Freshly prepared STZ solution (pH 4.5, 0.1 M cold citrate buffer) 45 mg/kg was administered as a single dose i.p. After 72 hours, blood glucose levels were measured. Animals with blood glucose values of more than 250 mg/dl were considered diabetic and included in this group.

Diabetes Mellitus + Barley Grass (DM+BG) group, a single dose of STZ solution (pH 4.5, 0.1 M cold citrate buffer) 45 mg/kg was injected i.p. After 72 hours, blood glucose levels were measured. Animals with blood glucose values of more than 250 mg/dl were considered diabetic and included in this group. Diabetic animals in this group were additionally given BG water (3 ml/rat/day) was given as gavage for 4 weeks.

Sample collection: At the end of the four-week experiment, the body weights of the animals were recorded and blood samples were taken from their hearts with a sterile syringe of all animals under anesthesia. Animals for anesthesia were given xylazine HCl (10 mg/kg), and ketamine HCl (70 mg/kg) as i.p.

Blood samples were quickly placed in tubes with anticoagulants. They were separated into serum by centrifugation at 1800xg (3000 RPM) for 10 minutes (+4 °C). The serums were stored in the freezer (-20 °C) until analysis.

Determination of glucose and HbA1c levels: These parameter levels were measured by the immuno-turbid metric method by the auto-analyzer (Modular P800i, Roche, Germany).

Determination of oxidative DNA damage: To determine DNA damage, the 8-hydroxy-2'-deoxyguanosine (8OHdG) value was measured. DNA damage was analyzed by ELISA kit (Enzo Life Sciences, USA).

This kit was worked on the Elisa reader and washer (Stat Fax, USA) according to the manufacturer's instructions. Other

technical data about the kit are as follows.

Measurement method: The Absorbance 96 is designed to carry out sensitive absorbance measurements. It measures the optical density (OD) of samples at defined wavelengths.

Measuring technique: Endpoint and Kinetic

Wavelength: 450 nm

Linearity: ≤ 1.5 % (0–2 OD), ≤ 3.0 % (2–3 OD)

Accuracy: ≤ 1.5 % + 0.010 OD (0–2 OD), ≤ 3.0 % + 0.010 OD (2–3 OD)

Reproducibility : ≤ 0.5 % + 0.005 OD (0.0–2.0 OD), ≤ 1 % + 0.005 OD (2.0–3.0 OD)

Measurement range 0–4.0 OD

Sensitivity: 0.59 ng/ml (range 0.94 - 60 ng/ml)

Determination of TOS, TAS and OSI levels: TOS and TAS values were determined by colorimetric kits (Rel Assay, Turkey) in blood serum. The oxidative stress index (OSI) was calculated with the ratio of TOS to TAS.

The TAS and TOS kits were studied in the spectrophotometer device (Shimadzu, Japan) according to the manufacturer's instructions. Other technical data about the kit are as follows.

Principle of TAS Assay: Antioxidants in the sample reduce dark bluegreen colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is a vitamin E analog .

Precision: Inter-assay coefficent of variation 2.8%, Intra-assay coefficent of variation 3.3%

Assay Range: 0.1 – 3.5 mmol Trolox Equiv. /L.

Wavelength: 660nm

Principle of TOS Assay: Oxidants present in the sample oxidize the ferrous ion– chelator complex to ferric ion. The oxidation reaction is prolonged by enhancer molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with chromogen in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H2O2 Equiv./L)

Precision: Inter-assay coefficent of variation 3.2%, Intra-assay coefficent of variation 3.9%

Assay Range: 0.2 – 80 µmol H2O2 Equiv. /L.

Wavelength 530nm

Determination of live weight: The live weights of the rats in the whole group were measured with a precision balance at the end of 4 weeks.

Statistical analysis: SPSS version 16.0 was used for statistical calculations. Statistical Data were given as mean±standard deviation (M±SD). The calculator was taken at a 5% significance level for analyses. The Kruskal-Wallis test was used to analyze all data and the Dunn test was used to identify different groups.

Results

Effect of barley grass on 8-OHdG level: Administration of barley grass to rats injected with STZ did not change oxidative DNA damage. Therefore, there was no difference in mean serum 8-OHdG levels between all groups (*p=0.059*) (Table I).

Effect of barley grass on TOS and OSI activities: Streptozotocin- intoxicated elevated serum TOS and OSI in the Diabetic animals compared to animals in the control and BG groups. TOS (*p=0.035*) and OSI (*p=0.004*) values of rats in the DM+BG Group statistically decreased compared to the DM group (Table I)

Effect of barley grass on TAS activities: barley grass-treated increased serum total antioxidant status in animal's BG and DM+BG groups compared to other groups (*p=0.005*) (Table I).

Effect of barley grass on glucose and HbA1c values: these levels were significantly elevated in the DM animals compared to the other groups. BG treatment in Diabetic rats significantly decreased glucose (*p=0.006*) and HbA1c (*p=0.005*) values. These values of rats in the DM+BG Group were statistically decreased compared to the DM group and these value in the BG Group were close to the control group. (Table I).

Effects of barley grass on live weight: Streptozotocin-intoxicated decreased live weight in the diabetic animals. Barley grass treatment in diabetic animals significantly increased these parameters. The live weight of rats in the DM+BG Group was statistically increased (p=0.041) compared to the DM group. These values in the BG group were close to the control group. (Table I).

Table I. The values of serum 8-OHdG, TAS, TOS, OSI glucose, HbA1c and live weight values in all groups

Parameters	Control (C) Mean±SD	Barley grass (BG) Mean±SD	Diabetes (DM) Mean±SD	Diabetes and Barley grass (DM+BG) Mean±SD	*p*
8OHdG (ng/mL)	13.35±6.25	15.55±8.18	18.98±8.23	17.38±6.57	*0.059*
TAS (mmol Trolox Equiv/L)	0.57±0.24^{b}	1.02±0.25^{a}	0.68±0.19b	0.97 ±0.38^{a}	*0.005*
TOS (µmol H_2O_2 Equiv/L)	4.15±1.13^{b}	5.27±1.63^{b}	8.12±1.72a	6.29±1.87^{ab}	*0.035*
OSI (Arbitrary Unit)	0.73±0.31^{b}	0.53±0.15^{b}	1.19±0.78a	0.65±0.65^{b}	*0.004*
Glucose (mg/dL)	78.25±5.57^{c}	91.53±8.45^{c}	482.00±69.58^{a}	349.00±52.76^{b}	*0.006*
HbA1c (%)	2.58±0.35^{c}	2.98±0.5^{c}	6.74±1.15^{a}	4.45±0.95^{b}	*0.005*
Live Weight (g)	270.60±20.10^{a}	284.35±18.30^{a}	202.75±15.70c	237,35±16.45^{b}	*0.041*

$^{a,b,c:}$ in the same line values with different letters show statistically significant differences.

^{c}: Control, BG: Barley Grass, DM: Diabetes Mellitus, 8-OHdG (8- hydroxy-2'-deoxyguanosine), TAS - Total antioxidant status; TOS - total oxidant status; OSI -oxidative stress index; HbA1c: Hemoglobin A1c

Discussion

Free radical formation is seen in the occurrence of some chronic diseases. Antioxidants that work against free radicals become increasingly important. Cytotoxic aldehydes are products of lipid peroxidation. It causes damage by attaching to DNA and proteins (20). In such cases, DNA integrity may be compromised and DNA damage may develop. 8-OHdG is a frequently used parameter in the determination of DNA damage (21).

In previous experimental studies, it was reported that oxidative stress due to diabetes causes DNA damage. As a result, it was emphasized that the level of 8-OHdG increased in the tissues and body fluids of these patients (22). In another study, it was revealed that an antioxidant-effective of lycopene (23) and wheatgrass (24) used reduced the level of 8OHdG and had a DNA protective effect. On the other hand, in some studies such as diabetes (10), and kidney failure (14,15) it was reported that DNA damage was not observed.

In this study, when all groups were examined, serum 8OHdG level was highest in the diabetic group, but this increase was not statistically significant. According to our results, we can say that short-term diabetes does not exactly cause DNA damage. Already, the 8OHdG level is compatible with the literature (10,14). In some cases, oxidative damage that occurs at low levels can be effectively repaired by metabolism, some enzymes involved in circadian control mechanisms (25). H2O2 does not directly damage DNA like O2, but OH is more effective on DNA (26). In addition, for OH radicals to have an effect on DNA, they must be formed either in DNA or very close to it.

Free radicals can damage tissues and some related diseases may occur. At the same time, free radicals can affect the nucleic acids of cells, resulting in cell death and premature aging. In addition, cells change to form cell lines that cause cancer and similar diseases (27).

Free radicals elevated in blood serum may cause membrane integrity loss and genetic mutations (8). Reactive oxygen species such as hydroxyl, superoxide anion, and oxygen radicals are believed to cause carcinogenesis, mutagenesis, aging, and arteriosclerosis. Endogenous antioxidants have been found to protect the body against reactive oxygen, but there is an exciting increase in the protective functions of natural antioxidants found in plants (28).

In other metabolic diseases such as diabetes mellitus, the main factor causing oxidative stress in cells is decrease in the amount of antioxidants and an increase in reactive oxygen species (ROS) is associated with hyperglycemia. Thus, hyperglycemia; in organs such as heart, kidney, eye, liver; It causes oxidative damage in the gastrointestinal tract, small and large vessels and nerve tissue (29). Studies have shown that free radical production increases in diabetes, resulting in delayed healing of wounds (10, 11).

Recently, serum TOS and OSI levels have been reported to be high in diabetes (10), kidney failure (14, 15), in methotrexate administration (19), cancer (16). This study, blood serum TOS and OSI values were found highest in the rats in the diabetes group. It was determined that these values decreased with the effect of barley grass and approached the values in the control group. In this case, we can say that barley grass has a healing effect on oxidative stress in diabetic rats and Barley Grass used in diabetes can reduce this increased oxidative stress state.

Antioxidants play a dual role by removing free radicals from cells, slowing and even preventing diseases (30). The reactive oxygen species activity may increase and the activity of the antioxidant enzymes may decrease (31). Some antioxidants have a protective effect on this toxicity, suggesting that these damages may be related to radical metabolism (32). Studies have shown that antioxidants prevent cell damage by neutralizing free radicals (27). Antioxidants in the body can partially protect the person against reactive oxygen species. It is necessary to take extra antioxidant supplements from outside. Antioxidants may be needed externally to prevent the damage of free radicals that increase diabetes (8).

In diabetes, glutathione reductase, catalase and glutathione peroxidase activities decrease (33). On the other hand, vitamins C and E reduce lipid peroxidation in diabetes (34). At the same time, vitamin E reduces fasting plasma glucose, fructosamine, thiobarbituric acid reactive substances (TBARS) and increases superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, insulin and C peptide levels, in Type 2 diabetes (35).

Some plants, such as barley grass, contain large amounts of antioxidant enzymes. There are high phenolic compounds with antioxidant properties in barley grass. These compounds are in the form of phenolic acids, proanthocyanidins, tannins, flavonols, chalcones, flavones and amino phenolic compounds, tocopherols, polysaccharide, dietary fiber, and phytic acid. The antioxidant activity of polyphenols in barley, in order from most to least, are flavanols, flavonols and hydroxycinnamic acids (36).

Barley grass reduces oxidants such as malondialdehyde (MDA) and glutathione (GSH), GSH-Px, SOD, catalase (CAT), etc. provides an increase in antioxidants (7). In previous studies, it has been reported that as a result of oral administration of barley grass extracts to 36 patients with type 2 diabetes for 4 weeks, 15 g per day, it reduces free oxygen radicals, preserves low density lipoprotein (LDL), Vitamin-E level, and reduces LDL oxidation (37). Another study determined that barley grass used in similar doses increased TAS levels (14).

Mis et al., (10) reported that TAS values in the wheatgrass group were higher than the diabetes group in their study. In this study, TAS level in rats with diabetes was found to be close to the values of the control group. In addition, TAS levels of the barley grass and diabetes barley grass group were found to be statistically higher than the control and diabetic group. These findings support the antioxidant content of barley grass.

Oxidative stress induced by diabetes leads to dysfunction of pancreatic B cells, glucose intolerance and insulin resistance. Phytochemicals such as phenolic acids, phytosterols, tocopherols and flavonoids in barley may be beneficial in treating of these diabetes disorders (38).

The treatment of diabetes aims to balance glucose homeostasis. For this reason, glucose production is reduced or insulin production is increased. In both cases, it is desired to reduce insulin resistance in the receptors of the relevant cells. For this purpose, many drugs and methods have been developed in the treatment of diabetes.

Barley contains important antidiabetic elements. These include β-glucans, phytosterols, phenolic compounds, tocopherols, resistant starches and arabinoxylans. β-glucan

in barley can reduce serum lipids, arterial sclerosis, serum glucose and insulin resistance in obese mice (39). Furthermore, due to β-glucan, long-term use of foods such as barley can improve insulin resistance and prolong the feeling of satiety (40). Slow-digesting starch and High phenolic content in barley cakes can improve the glycemic pathway (41).

In previous studies (42) the alcohol extract of barley was administered to diabetic rats at different doses for 11 days, and their blood glucose was monitored daily. A significant decrease in blood glucose in diabetics was determined with barley applications at doses of 250 and 500 mg/kg. In addition, it has been stated that if barley is applied for 4 weeks, it can eliminate these negative effects in diabetics by showing hypoglycemic and antioxidant effects (43).

In this study, it was determined that the glucose level, which was high in the diabetes group, decreased with barley grass application and this change was quite significant. This shows that barley grass is hypoglycemic and can be used therapeutically in diabetes patients.

Hemoglobin A1c (HbA1c), known as glycated hemoglobin, is a compound formed by hemoglobin with glucose and its amount varies depending on glucose concentration. HbA1c is a parameter that shows the glucose level for 8-12 weeks. It is used as a biomarker for complications that may develop in diabetes because it shows long-term glucose levels. HbA1c is required for long-term glucose control retrospectively in patients with diabetes. Therefore, it is widely used. In diabetes, the HbA1c level increases as a result of the reaction of high blood sugar and hemoglobin (44). In this study, the level of HbA1c was significantly higher in diabetic rats. A decrease in HbA1c level was observed with barley grass application. The results obtained were consistent with the literature (9).

Diabetes can cause bodyweight loss, increased fat and protein catabolism, and destruction of muscles . With the decrease in insulin secretion in the body, the peptide bonds of proteins are hydrolyzed, and broken down into peptides and amino acids. As a result, muscle tissue weakens (45). In another study, approximately a 35% increase in body weight in diabetic animals compared to the healthy group (9, 46).

In this study, the live weights of the animals in the diabetic group were found to be quite low. On the other hand, there was a gain in the live weight of the animals in the DM and BG Groups. It can be said that the blood sugar levels of rats may decrease due to barley grass extract given to the diabetic group. As a result, it should be considered that weight loss is less due to the decrease in lipolysis and proteolysis.

There are also studies showing the curative effect on obesity of barley. β-glucan found in barley plays a role in obesity treatment by increasing flow-mediated expansion by reducing serum p-cresyl sulfate, total cholesterol and low-density lipoprotein levels (47). β-glucan in barley, which improves food digestibility and adds antiobesity, can prevent obesity of visceral fat and increase stool score (48). Insulin resistance and obesity are associated with bile acid changes with low dietary fiber in the barley diet (49). Barley malt has an antiobesity effect thanks to the β-glucan and phenolic acids it contains (47).

Diabetes Mellitus disease, which is becoming more common day by day, has an increasing prevalence. Many mechanisms play a role in the pathogenesis and complications of the disease. The most accepted one is related to free radicals. By increasing the antioxidant capacity, oxidative stress caused by diabetes can be dealt with. Studies on the effects of antioxidants on diabetes are increasing.

Some studies have found that barley grass extract reduces oxidants such as MDA and increases antioxidants such as CAT, SOD, GSH and GSH -Px. However, according to our literature review, there is no published report on the effect of barley grass on TOS, TAS, OSI, DNA damage and serum oxidative stress parameters such as glucose and HbA1c in diabetic rats. Therefore, this study is important.

With this study, we revealed that the antioxidant defense system is strengthened by barley grass. In addition, it caused a decrease in glucose levels and body weight in diabetic rats. In other words, barley grass may contribute to diabetes management by reducing and preventing diabetic complications. For this reason, we recommend barley grass to beat oxidative stress in diabetic patients. We think that barley grass will also be beneficial due to its anti-oxidative, anti-hyperglycemic, and weight effects.

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